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14. ABSTRACT

Oxidative stress may play a role in human oncogenesis, including breast cancer. The mitochondria are most common sources of reactive oxygen species (ROS) responsible for most oxidative stress. This project evaluates the role of mitochondrial abnormalities in oxidative stress in breast cancer development. Complex II mutant subunits, affecting the quinone-binding site or the heme b ligand binding site, were generated and analyzed in both cell culture and transgenic mouse systems in terms of mitochondrial functions, ROS production and oncogenesis. Results from this study demonstrate that short-term expression of these mutants induces apoptosis in the cells; and transgenic mice harboring these mutant complex II subunits develop tissue vascularization and tumors at advanced ages. These findings suggest a role of oxidative stress in breast cancer development and progression, and provide clues on whether antioxidants are beneficial in prevention and treatment of such important cancer in women.

15. SUBJECT TERMS
reactive oxygen species, mitochondrial complex structure, WAP promoter gene activation, transgenic model, breast cancer

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INTRODUCTION

Breast cancer is the most frequently diagnosed cancer and accounts for >15% of cancer death, only second to lung cancer, among women in America (23). Although recent biomedical advances have identified several genetic components whose mutations contribute to the multi-step oncogenesis of the breast. These genes include the tumor suppressor genes, BRCA1 and BRCA2 that account for less than 5% of all breast cancers (39). Although germ-line mutations in the p53 tumor suppressor gene are associated with the early onset breast cancer in the Li-Fraumeni syndrome (15) and amplification of the proto-oncogene, ERBB2/NEU is frequently observed in breast cancer (19,45), the origins of sporadic breast cancer are still largely unknown.

Mammary oncogenesis is influenced by various environmental agents and multiple genetic components. An accumulation of additive mutational events is needed for either the initiation or predisposition of this organ to oncogenesis (21). Among the numerous external agents and internal factors, oxidants in the forms of reactive oxygen species or ROS (superoxide ($O_2^{\bullet -}$) hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\bullet}$)) have been suspected to contribute to the mammary tumorigenic process (6,14,22,25,37,44). Several nutritional and population studies suggested that antioxidant intake may lower the risk of breast cancer (1,10,29). Other clinical and laboratory investigations showed increases in ROS concentration in breast tumor tissues/cells compared to those in normal controls (18,34,35). Accumulation of harmful levels of ROS in the cells impairs cellular functions by abnormal lipid peroxidation and protein oxidation and exerts long-term mutational events on the DNA by adduct formation. Despite such associations, ROS as etiologic agents for breast cancer have not been well-established in animal models. Mitochondria serve numerous essential cellular functions of energy production, ion balances, and regulation of apoptosis (38,48). They are also important sources of cellular ROS as by-products of their energy production through oxidative phosphorylation (OXPHOS). In this process, electrons are donated by carbohydrates and fats by enzymatic activities of mitochondrial Complex I and II which are then passed on to the remainder of the Complexes III and IV, resulting in the synthesis of ATP from ADP at Complex V. The electron transport chain (ETC) through the mitochondrial Complexes is the central energy transport mechanism in OXPHOS. Currently, the exact source(s) of ROS from mitochondria is still uncertain. Recent advances in atomic modeling of the mitochondrial Complex II and discovery of mutations on genes coding for the subunits of this Complex responsible for the hereditary paraganglioma and pheochromocytoma (2-4,12,13,30,31,32) suggest that Complex II may also play a critical role in ROS homeostasis, oxygen sensing (11,26) and hypoxia response (5,40,41). These new findings, described below, have offered a unique opportunity to investigate the link among mitochondrial structure, ROS production, and human oncogenesis.

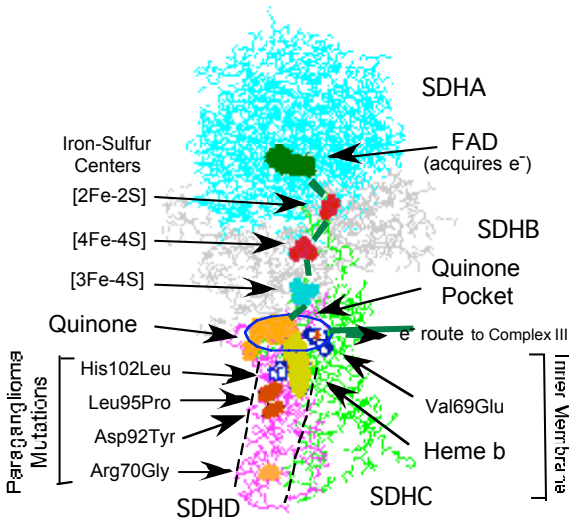

Figure 1. Structure of mitochondrial complex II. Paraganglioma mutations are primarily located on the second trans-membrane helix of the D subunit. Val69Glu mutation corresponds to pre-mature aging mutation in C. *elegans*.

Complex II constitutes the

succinate:quinone oxidoreductase (or succinate dehydrogenase, SDH) (38). It is the smallest Complex of the mitochondria. It is composed of 4 subunits called A, B, C and D that are encoded by 4 nuclear genes, designated as SDHA, SDHB, SDHC and SDHD respectively (Figure 1). SDHA is a flavin-binding protein and contains the active site for the enzyme. SDHB is an iron-sulfur protein containing 3 iron-sulfur centers. SDHC and SDHD are two small subunits anchoring the entire Complex on the inner membrane of the mitochondria. SDHA converts succinate to fumarate and generates electrons that are trapped by the flavin (FAD) and then channeled through the iron-sulfur clusters in SDHB and finally to the ubiquinone at a pocket, called quinone pocket, formed by the two anchor subunits and the iron-sulfur protein (7-8,17). The quinone then takes the electrons (being reduced), migrates and passes them on to Complex III. SDH is the only membrane bound member of the TCA (Kreb's) cycle and is the direct link between ETC and TCA cycle. Complex I, or NADH ubiquinone oxidoreductase, reduces NADH and transfers electrons similarly to ubiquinone and the remainder of ETC in OXPHOS. It is the largest Complex in the mitochondria with >42 subunits, 7 of which are encoded by the mitochondrial genome (38). Its exact structure at atomic level has not been elucidated. Since NADH is synthesized in the TCA cycle, SDH also indirectly controls the supplies of substrates to Complex I. Both Complex I and II are the sole sources of electrons in the ETC. Hence defects on Complex II will reduce electron supply at both sources for OXPHOS, impair energy production and slow down metabolism in the TCA cycle.

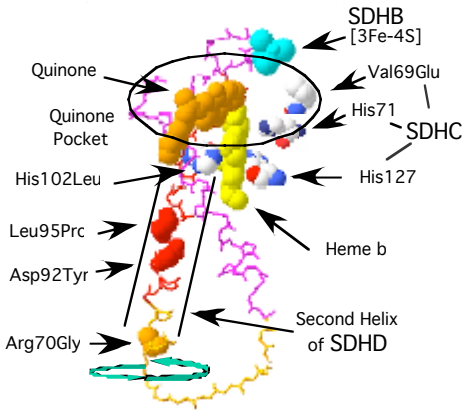

Figure 2. Illustration of paraganglioma mutations affecting the structural integrity of the second helix. Val69 and His71 on C subunit occupy critical entry sites at the quinone pocket.

Hereditary paragangliomas are vascularized, neural crest-derived tumors of the sympathetic paraganglia, primarily located in the carotid body at the base of the skull or middle ear. Disease is usually inherited paternally with the simultaneous loss of the maternal allele exclusively in the tumor (5). Recent studies identified mutations at genes coding for the B, C and D subunits of Complex II to be responsible for the disease (see review 4). In particular, SDHD gene seems to harbor the most mutations in which a mutant protein is synthesized in each case. These findings have led us to embark on a detailed analysis of the atomic structures of succinate dehydrogenase and its anaerobic homologue, fumarate reductase, from E. coli (7,43). The bacterial succinate dehydrogenase contains 4 subunits and is structurally homologous to the mammalian Complex II with conservation of all critical residues (17,43). Our results suggest that mutations on SDHD would have imposed structural constrains on its second trans-membrane helix, affecting the heme ligand binding activity (Figure 2). One particular mutation, His102Leu, disrupts the SDHD ligand binding site for heme b. The second heme binding site is at His127 of SDHC, the other anchor subunit. Both ligand binding sites form covalent hydrogen bonds with the iron (Fe) molecule in the center of heme b through their respective imidazole ring (Figure 3). Replacing a positively charged His residue with a neutral

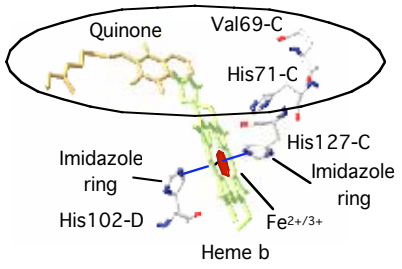

Figure 3. His102 on the D subuni and His127 on the C subunit are the ligand binding sites for heme b. These residues bind to the Fe ion through their imidazole ring.

Leu residue will abolish the ligand binding at SDHD. It can destabilize heme b and/or allow other His residue(s) to bind to the iron molecule (7,28). Similar mutations at the analogous residue of the bacterial enzyme resulted in lowering of the heme b redox potential by 100 mV leading to a dramatic reduction (>90%) of the enzymatic activity and a significant increase in ROS production (27). Other mutations on SDHD, identified on paraganglioma patients, also disrupt the second trans-membrane helix and lead to structural distortions. We believe such structural instability caused by the various mutations will not only affect the redox potential of the heme b but also the adjacent quinone pocket and the rest of the ETC apparatus (including the iron-sulfur clusters and FAD in the B and A subunits respectively). This, in turn, leads to semiquinone (Q^{-}) build up and ROS production. Under normal conditions, ROS are removed by scavenger enzymes, such as MnSOD, catalase and glutathione peroxidases, to reach a physiologic equilibrium or homeostasis (48). The cell might use such ROS homeostatic balance as one of its oxygen sensing mechanisms (9,11,26). The mutations in the paraganglioma create a homeostatic imbalance, leading to abnormal oxygen sensing by the carotid body that responds, as if hypoxic conditions exist, by tissue vascularization, cell proliferation and tumor growth in the absence of a real hypoxia.

The overall goal of this project was to address the question of oxidative stress, mediated by effects of mitochondrial Complex II mutations, in tumorigenesis, particularly on breast cancer using both cell culture and transgenic mouse strategies.

BODY

Task 1. To construct and characterize mutant transgenic mice

The Cre-LoxP Transgene Activation Strategy

As illustrated both in the original application and the first annual report, the Cre-LoxP transgene activation (16, 46,47) was initially adopted for the present study to target the expression of a mutant Complex II subunit in the mammary glands of transgenic mice. This system consists of two components, an activator that harbors a Cre recombinase transgene directed by a mammary gland specific promoter, such as the whey acid protein (WAP) or MMTV promoter, and a responder that harbors a bicistronic transgene directed by a strong actin promoter. Independently, these two transgenes will not produce any mutant Complex II subunits. However, when they are present in the same transgenic mice, the expression of the Cre recombinase will recombine the responder gene, thereby placing the Complex II mutant directly under the regulation of the strong actin promoter, resulting from a switch of transgene expression from the β-galactosidase to mutant Complex II subunit and an indicator molecule, the green fluorescent protein (GFP).

We have generated all the mutant Complex II subunit genes, i.e. V69E on subunit C and H102L in subunit D using site-directed mutagenesis. They were inserted into the responder vector, PCCALL2-IRES-EGFP (33), and used for transgenic mouse construction. For the mSdhC-V69E mutant, we have generated a total of 16 founder animals. To identify those with functional transgenes, we analyzed the expression of the β-galactosidase (= lacZ) gene and identified 3 transgenic lines that express the transgene. For the mSdhD-H102L Complex II

mutant, we obtained 6 founder animals. These transgenic lines were then crossed with non-transgenic mice to establish the respective transgenic lines.

For the activator lines, we had obtained transgenic lines, WAP-Cre (47) and CAG-Cre (16) from the Jackson Laboratory and the Mouse Models of Human Cancer Consortium at the National Cancer Institute through the Material Transfer Agreement Process. Due to the patent held by the Dupont Corporation on the Cre-LoxP system, negotiation was conducted by the Regional Counsel Office at the Veterans Affairs Medical Center, San Francisco. The entire process took approximately 4 months, after which breeder pairs of the respective transgenic mouse lines were transferred to the Animal Care Facility at the VA Medical Center, San Francisco. They were finally integrated into the breeding facilities after a quarantine period of two months at the isolated quarter.

Task 2. To correlate Complex II mutant expression to ROS synthesis and mammary oncogenesis in transgenic mice

The availability of the various activator and responder transgenic line now allows us to proceed with the assembly of the transgene activation system by crossing these transgenic lines to generate bi-transgenic mice. To facilitate such study, we initiated a breeding program to establish breeding colonies for both WAP-Cre and CAG-Cre mice. Once sufficient animals are available, they will be used in crossing experiments to the appropriate responder lines, harboring the mSdhC-V69E and mSdhD-H102L mutant Complex II transgenes.

To test whether any of the responder lines are capable of responding to a Cre mediated recombination, we have initiated crossing of breeding mice from Jackson Laboratory and the Mouse Cancer Model repository at NCI, particularly the CAG-Cre and another transgenic line, TSPY-Cre, generated in our own laboratory. The objective of these preliminary studies is to identify those functional responder lines for experiments to be conducted at a later stage with the mammary gland specific Cre recombinase lines. These experiments have just begun and, so far, we have obtained 2 double transgenic mice harboring both TSPY-Cre and mSdhD-H102L transgenes. The recombination status of their responder gene is currently being investigated.

Low Levels of Mutant Complex II Transgene Expression in Founder Animals

As discussed above and in previous annual report, we have generated 16 founders for the floxed mSdhC-V69E and 5 founders for the floxed mSdhD-H102L responder constructs. Of these, only a selected number of founder animals were capable of transmitting their transgenes to their offspring. Most transgenic founders are in the FVB genetic strain background. As these founders aged, we observed a series of abnormalities among those harboring the mSdhD-H102L construct, but not those harboring the mSdhC-V69E transgene or 6 additional founder animals harboring another transgene, TSPY, generated in our laboratory on another project. We surmise that these abnormalities might be related to low levels of the mutant mSdhD-H102L transgene expression, due to the leakiness of the gene construct. The PCCALL2-mSdhD-H102L-IRES-EGFP expression vector (Figure 4) is directed by a strong actin promoter that is ubiquitously active in most cell types.

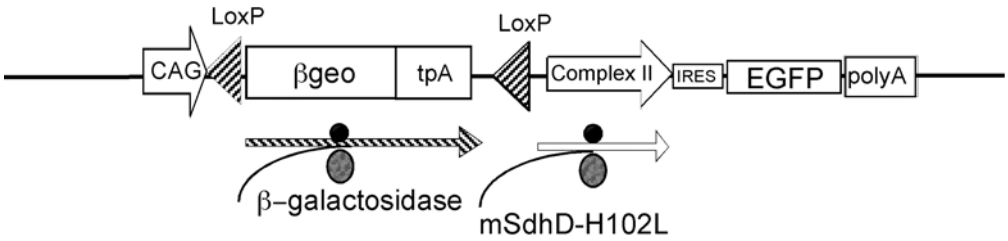

Figure 4. PCCALL2-mSdhD-H102L-IRES-EGFP transgene construct

Such high levels of tri-cistronic transcripts from this transgene might allow a low level of translational initiation at the coding sequence for the mSdhD-H102L, thereby expressing low levels of this mutant Complex II subunit. We postulate that a chronic expression of this Complex II mutant might have resulted in unintended consequences manifested phenotypically.

Analysis of recombination of *Lox-mSdhC^{V69E}* mice.

Five animals out of 13 founder animals of *Lox-mSdhC^{V69E}* represent β-galactosidase activity in tissues, indicating that the transgene was active in these animals. Three lines (lines 15, 45 and 56) were established from these 5 founders. These lines were mated with Cre-recombinase expressing mice, e.g. *TSPY-Cre* mouse (20) and *WAP-Cre* mouse (47), to stimulate recombination in the target tissues. *TSPY-Cre* mouse expresses Cre-recombinase in germ cells and neurons. *WAP* (Whey Acidic Protein) gene promoter is specifically active in alveolar epithelial cells of mammary tissue during lactation (42), and therefore the *WAP-Cre* mouse expresses Cre in alveolar epithelial cells of mammary tissue (Figure 1B). Although we were able to observe selected cells in the mammary gland of double-transgenic mice expressing the reporter EGFP, we were not able to observe extensive expression of the Complex II mutant in double transgenic mice harboring both the *Lox-mSdhC^{V69E}* and WAP-Cre transgenes, so far. These problems could potentially be attributed to the integration sites of the transgenes, the effectiveness of the Cre recombinase in cleaving the *loxP* sequences. Indeed, recently, others have reported that pronuclear injection might result in multiple-copy integration of the responder (*loxP*-tagged) transgene. Multiple-copy integration may cause undesirable number of *loxP* sites and possible chromosomal instability (24). We surmise that it might be the cases for the *LoxP-mSdhC^{V69E}* transgenic mice, thereby resulting in efficient activation of the Complex II mutant transgene in the double transgenic mice, observed in our studies.

Analysis of abnormal observation with *LoxP-D^{H102L}* mice.

The most prominent abnormality is the development of benign tumor-like mass on the flanks of 3 founding animals harboring the mSdhD-H102L transgene (Figure 5) at 8-12 months of age. Dissection of the tumors showed that they contained fatty masses, resembling tissue vascularization. Such tumor-mass disappeared when the transgene of one (#5 founder) of these 3 founders was introduced into a CD-1 background, suggesting that the FVB genetic background is important for its manifestation. The transgenes in the remainder two founders could not be transmitted to their offspring.

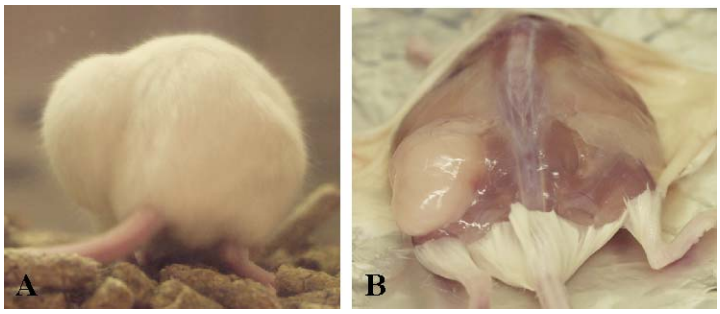

Figure 5. Tumor-like mass on the flanks of mSdhD-H102L responder transgenic founder

Only one line of *LoxP-mSdhD*^{H102L} (line 5, established from founder ID#5) represented the β-galactosidase activity in tissues. This line also could not be stimulated recombination in double transgenic mice with Cre-expressing lines. However, unexpectedly, one female mouse (15 month old) formed tumor in vagina and uterine cervix without Cre dependent recombination (Figure 6A and 6B). Some metastatic tumors were also observed in kidney (Figure 6C) and liver in this animal (Figure 6D). In addition, another founder female animal (ID#6, 17 month old) formed tumor-like structure in left lung (Figure 7A and 7C). In this animal, the transgene seemed to be active in mosaic manner, and only tumor-like cells of lung showed β-galactosidase activity (Figure 7E, arrows). Since the *CAG* promoter is extremely strong promoter and the βgeo gene was flanked by possible alternative-splicing sites, we surmised that leaky expression of SdhD^{H102L} might have caused abnormal cell growth without Cre-mediated recombination. These preliminary results suggest that this Complex II mutation could potentially exert oncogenic effects on its host animals. We were not able to observe mammary gland-specific activation of the transgene after crossing with the WAP-Cre transgenic line (Figure 8), however the levels of transgene expression was relatively low. Based on these findings, we have re-designed our strategy, as discussed in the previous report, and initiated additional studies to generate transgenic mice harboring a mammary cell specific *mSdhD*^{H102L} transgene.

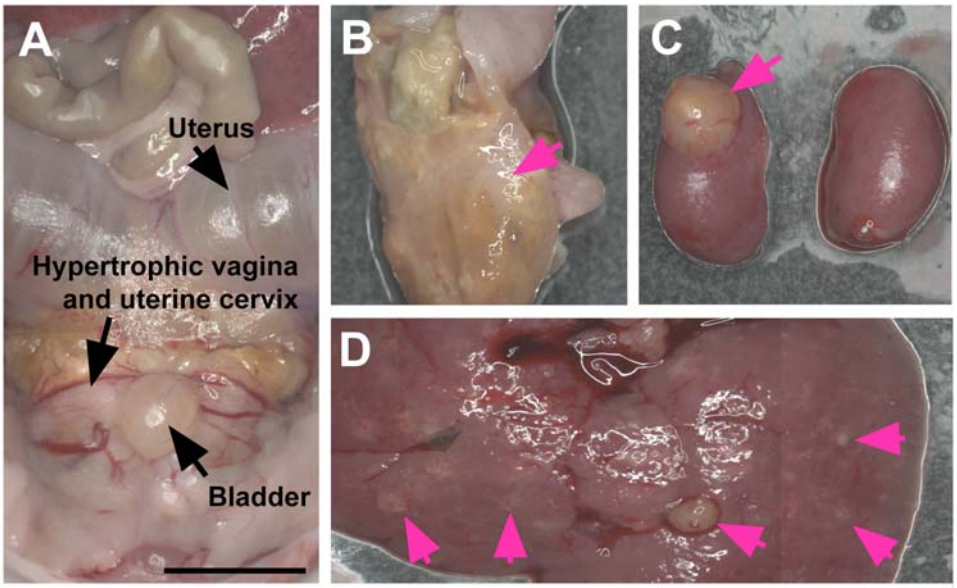

Figure 6. Tumor formations in a female *LoxP-mSdhDH102L* mouse (line# 5, 15 month). A) Intraperitoneal image showing uterus, uterine cervix and vagina. Uterine cervix and vagina were abnormally hypertrophied, and uterus was filled with fluid. B) Cross section of vagina and uterine cervix. C) Metastatic tumor formed in kidney. D) Morphology of liver showing abundant white dots were observed in (arrows). Scale bar = 10mm in A-C, 83mm in D.

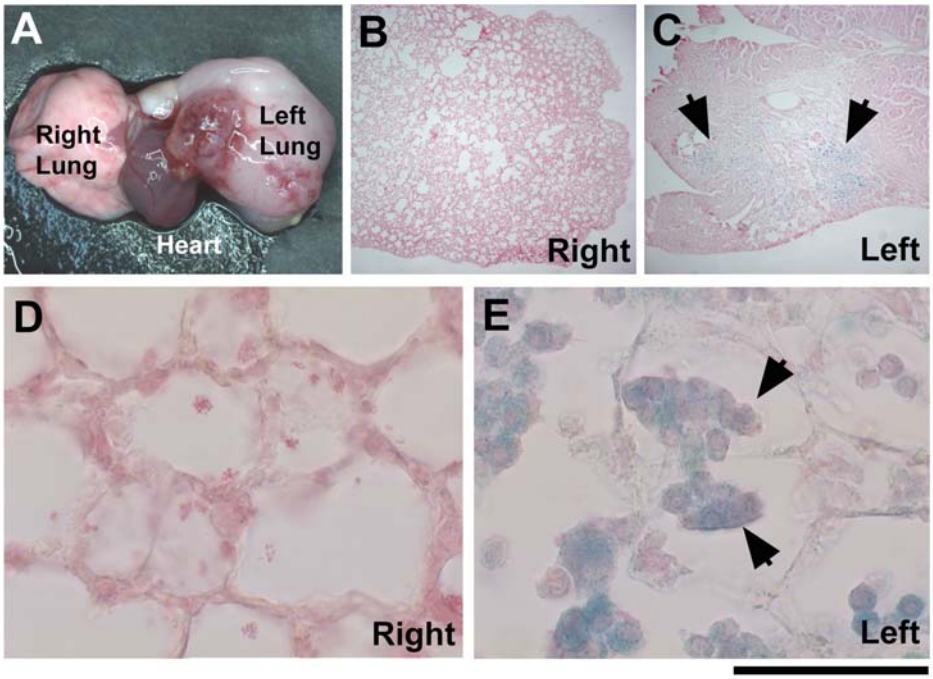

Figure 7. Hypertrophic lung in a female *LoxP-mSdhDH102L* mouse (ID#6, 15 month). A) Image of lung and heart. The left lung was bigger and whiter than the right lung. B and C) X-gal stained sections of right lung (B) and left lung (C) at low magnification. β-galactosidase positive cells were stained blue (arrows). Sections were counter stained by Nuclear Fast Red to visualize nucleus (red color). D and E) High resolution images of B and C, respectively. Blue cells, β-galactosidase positive cells, were filling the pulmonary alveolus in left lung (arrows in E). Scale bar = 100μm in D and E.

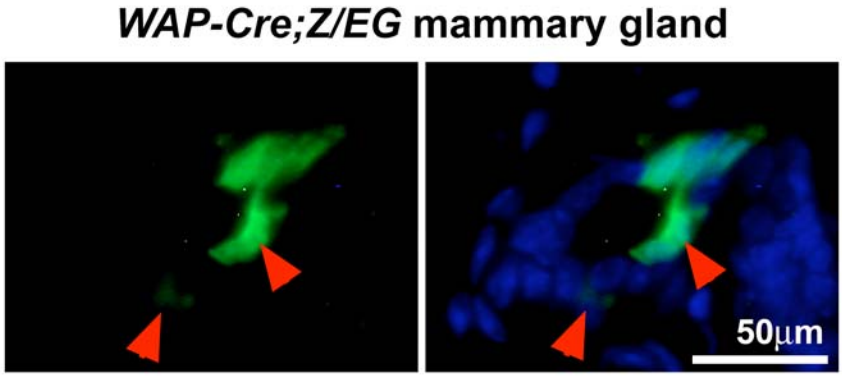

Figure 8. In double transgenic mice harboring both *LoxP-SdhCV69E/DH102L* and Cre expressing transgenes, Cre recombinase cleaves the *βgeo* gene flanked by *LoxP* sites, thereby *SdhCV69E/DH102L* and *EGFP* genes are expressed under the control of CAG promoter. Left, image of EGFP (green fluorescence) expression in mammary gland of *WAP-Cre;Z/EG* double transgenic mouse. *Z/EG* mouse harbors the reporter transgene expressing βgeo before recombination, and expresses EGFP after Cre-dependent recombination. Thus the EGFP expression corresponds to tissue-specific (i.e. mammary) activity of the WAP promoter in *WAP-Cre;Z/EG* double transgenic mouse. Right panel shows a merged image of EGFP (green) and DAPI staining for DNA (blue).

Refinement of the Transgenic Mouse Strategy

The high frequency of abnormalities among the founders of mSdhD-H102L transgene suggests that a leaky expression of this mutant Complex II, abide at extremely low levels, could have effects on the host animals. To seek confirmation on such postulation, we need to address the possibility of the mutation H102L in the mSdhD subunit of Complex II could be mutagenic in these processes. Further, we plan to determine if a chronic expression of this mutant subunit disrupts the Complex II structure and functions, thereby elucidating a certain level of oxidative stress in the affected tissues. Ultimately, we like to target such mutant to the mammary glands of the transgenic mice and evaluate its effects on breast cancer development, the initial goal of this Idea project.

Currently, we believe that the Cre-LoxP transgene activation strategy should still be a definitive study to establish the role of mutant subunit Complex II, particular the mSdhD-H102L mutation, in the oncogenic process using transgenic mouse technique. However, given the sophistication of the strategy and the time-consuming nature of transgenic mouse breeding and operation, this objective might not be achievable within the time frame of this project. To quickly address the importance of the mSdhD-H102L mutation in Complex II structure and function, and its relationship to oxidative stress and oncogenesis in the mammary glands, we plan to simplify our transgenic mouse strategy and to target the expression of such a mutant subunit in the mammary glands by a tissue-specific promoter, the whey acidic protein (WAP). We surmise that WAP promoter has been used extensively and successfully in targeting the expression of other oncogenic genes in the mammary glands of transgenic mice that eventually develop breast cancer. Since generation of oxidative stress in the mammary glands might not be detrimental to the host animals, we should be able to observe the effects due the expression of the mSdhD-H102L mutant in breast cancer development and progression.

To accomplish this goal, we have obtained a WAP-expression cassette consisting of 2.42-kb of WAP gene 5'-flanking sequence and 4.6-kb of WAP-3'-flanking sequence from Dr. Eric Sandgren via Dr. Jeffrey Ross, University of Wisconsin. Previous studies have demonstrated that insertion of a mini-gene in between these two WAP gene sequences resulted in high expression of the inserted gene in the mammary glands of the transgenic mice (36,42). To generate a mSdhD expression cassette, we inserted either the mSdhD-H102L or the wild type coding sequence, followed by an IRES (internal ribosome entry site) and the reporter gene, EGFP, between these WAP 5' and 3' flanking sequences (Figure 8). Both the wild type and the normal and mutant mSdhD subunits are tagged with the V5 epitope, so that the products of the transgenes can be distinguished efficiently from that of the host endogenous gene. Again, the IRES and EGFP sequences will provide a means to observe the transgene expression by detecting the EGFP fluorescence without sacrificing the host animals. We are currently generating transgenic mice harboring this mutant mSdhD-H102L construct.

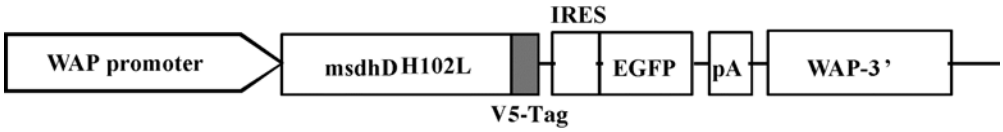

Figure 9. WAP-promoter directed mSdhD-H102L mutant construct targeting mammary gland transgene expression. A V5-epitope tag is fused with the mSdhD gene to distinguish the products between the transgene and the endogenous mSdhD gene. EGFP is included as a reporter for visualization of transgene expression in live animals.

Characterization of mSdh mutant subunits in cell cultures

To test the feasibility of using a small V5-tag as a means to detect transgene expression in the mitochondria, the mSdhD-H102L-V5 mutant and wild type-V5 cDNAs were expressed in COS7 cells under the CMV promoter using transient transfection technique. The transfected mSdhD products were detected by immunofluorescence (red) while the mitochondria were visualized by MitoTracker green procedures. Our results demonstrated that the V5 is efficient in tagging the recombinant products from transfected mSdhD genes using immunofluorescence and that the mSdhD protein is co-localized in the mitochondria of the transfected cells (Figure 10).

Generation of *WAP- DH102L-V5tag/EGFP* mice

Since we encountered several difficulties in the transgene activation in our double-transgenic mice with the Cre/loxP system, we have decided to directly express the mutant mSdhDH102L Complex II subunit in the mammary glands of transgenic mice directed by the WAP promoter. To establish these lines, we have performed further microinjections and generated the *WAP-msdhDH102L-V5tag/EGFP* mice to study the effects of this mutant on mammary grand (Figure 9). This mutation is selected because we observed numerous tumorigenic events among the hosts of the *LoxP-mSdhDH102L* transgene, as described above. The *WAP* gene promoter is specifically active in alveolar epithelial cells of mammary tissue (ref 42 and Fig. 8), thereby should be able to direct the mutant transgene expression in the mammary target cells. We have also incorporated the epitope V5 as a tag for the SdhDH102L mutant, thereby allowing a more efficient means to detect the expression of this mutant transgene. Again, we have also included the fluorescent biological marker, EGFP, for convenient in vivo imaging of the tissue-specific transgene expression. Our efforts, so far, have produced 11 male and 11 female founder animals harboring transgene. Currently, these founder animals are mated with non-transgenic mice to form transgenic lines and to analyze transgene expression in mammary gland.

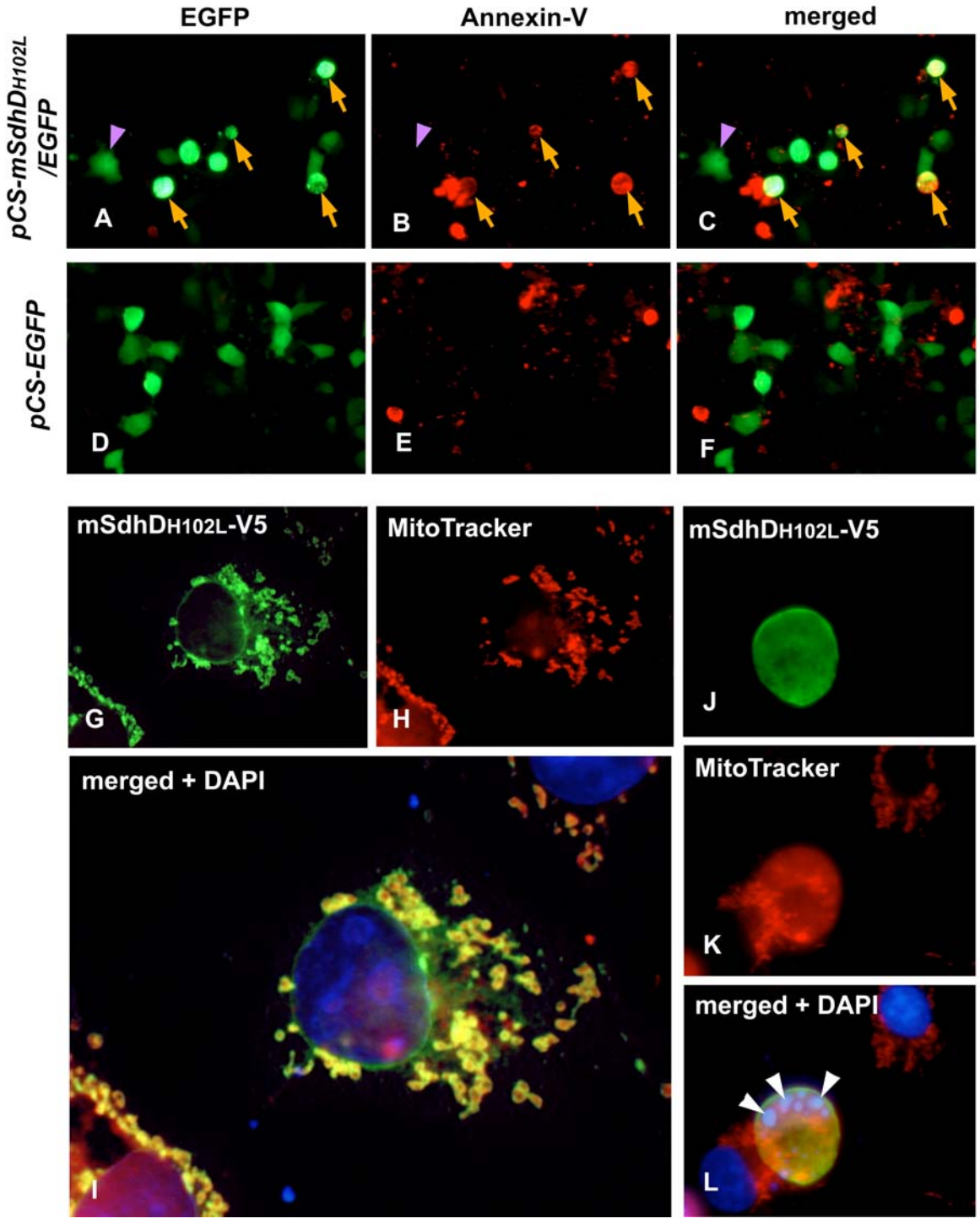

Figure 10. Expression of mutant mSdhd-H102L induces apoptosis in cells. An epitope-tagged mSdhd-H102L mutant-EGFP transgene was expressed in COS7 cells. Forty-eight hours after transfection, numerous cells with EGFP expression (A) showed high apoptosis, as detected annexin-5 immunofluorescence (red) (B, orange arrows), as in merged image (C, orange arrows) while cells transfected with vector alone (D) did not show any apoptotic activities (E, F, merged image). The expressed mSdhD-H102L products (G, green – detecting V5 epitope) co-localized with the mitochondrial tracking dye (H, red), as revealed on merged image (I). Many of the apoptotic cells

showed round up morphology and co-localization of transgene products (J) and disrupted mitochondria (K), while merged image showed fragmented nuclear materials (L, blue, white arrows).

Preliminary analysis of the transgenic mice showed that at least 5 founders were able to transfer their respective transgenes to their offspring. Transgene expression was detected in the mammary glands of selected animals. However, as of now, we are unable to observe any mammary gland tumors in these animals. These results, however, have to be considered in the context of results from previous transgenic mice in which tissue vascularization was observed in old (12-18 months of age) and the genetic background played a significant role of the tumorigenic outcomes. In particular, WAP gene is activated after pregnancy and lactation activities. Hence, the transgene expression could be heavily dependent on these physiological changes that require quite lengthy experimental and/or incubation period. We are currently maintaining these transgenic mice and waiting for future funding to continue the research.

KEY RESEARCH ACCOMPLISHMENTS

- Construction of transgenic mouse lines harboring mutant Complex II mutant expression cassette
- Analysis of mammary gland-specific Cre recombinase and Complex II mutant double transgenic mice
- Characterization of founder animals harboring mutant Complex II mSdhD-H102L transgene, demonstrating the potential involvement of this mutant gene in tissue vascularization and oncogenic lesions
- Refinement and simplification of the transgenic strategy to demonstrate the significance of the mSdhD-H102L mutation in mammary oncogenesis
- Successful generation of transgenic founders harboring a mammary gland-specific SdhD^{H102L} transgene

REPORTING OUTCOMES

None.

CONCLUSION

We have made good progress in evaluating the Cre-loxP strategy for tissue-specific transgene activation for the studies proposed in the original application. We have demonstrated the potential significance of a mutant Complex II subunit, mSdhD-H102L, in tissue vascularization and oncogenesis. Unfortunately, we were not able to observe specific mammary gland tumors develop in the recently generated WAP-mSdhd-H102L-EGFP transgenic mice. As in the case of the CAG-mSdhD-H102L transgenic mice, we only observed tissue vascularization and tumorigenesis in these animals at relatively old age. As intrinsic problems associated with transgenic mouse studies, we did not have sufficient time to manipulate and vary conditions of these animals to fully address the issues of oxidative stress in breast cancer. We believe future

studies of these transgenic mice should provided significant insights on the role of mitochondrial abnormalities and dysfunction in oxidative stress and mammary oncogenesis.

SO WHAT

Oxidative stress has been implicated in the etiologies of numerous human diseases. Successful implementation of the proposed research will provide critical insights on its role(s) in breast cancer. The availability of experimental animal models of breast cancer, pertaining to mitochondrial structural abnormalities and oxidative stress (8), will be important in understanding the disease mechanisms, potential prevention and therapeutic intervention for this devastating human cancer.

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